

## CLAIMS:

1. A process for producing recombinant calf-chymosin which comprises the steps of isolating calf-chymosin gene, cloning the same in bacterial expression vector PET21b, transforming said cloned vector into cells of E.coli, fermenting said E.coli strains to produce pro-chymosin, converting said pro-chymosin to chymosin and subsequently recovering the recombinant calf-chymosin.
2. The process as claimed in claim 1, wherein calf-chymosin gene is obtained by isolating RNA from the fourth stomach of calf tissue, synthesising a first strand of cDNA therefrom by treating the same with a reverse primer such as 5'-TGT GGG GAG AGT GAG GTT CTT GGT C-3' and then with a forward primer such as 5'-ATG AGG TGT CTC GTG GTG CTA CTT 3 and with a reverse primer such as 5'TGT GGT GAC AGT GAG GTT CTT GGT C-3'.
3. The process as claimed in claims 1 and 2 wherein said C DNA is ligated at small site of pBSSK+ plasmid and then transformed into TOP 10 cells of E-coli.
4. The process as claimed in claim 3 wherein said recombinant clones were identified and treated with a forward primer such as 5'-GAT ATA CAT ATG GCT AGC ATC ACT AGG ATC CCT CTG TAC 3' and reverse primer such as 5' GCA GTA AGC TTG ACA GTG TTC CTT GGT CAG CG-3' containing Nde I and Hind III sites to obtain an amplified fragment.
5. The process as claimed in claim 4 wherein said amplified fragment is transformed into cells of E.coli for expressing said chymosin gene.

6. The process as claimed in any of the preceding claims wherein said E.coli cells containing recombinant calf chymosin gene is fermented in a medium containing 12g/L peptone, 24g/L of yeast extract and 10g/L of sodium chloride in the presence of supplements for fermentation and the suspended cells produced on completion of fermentation is lysed, chilled and pH adjusted to 8 before incubating at room temperature and the supernatant containing prochymosin is separated.
7. The process as claimed in claim 6, wherein the pH of said prochymosin containing supernatant is adjusted to 2 at room temperature and further incubated for about 6 hrs with gentle stirring and filtered.
8. The process as claimed in claim 7 wherein the pH of said filtrate is adjusted to about 5 and further incubated, filtered and treated with a solution containing sodium benzoate and thereafter a solution containing and sodium chloride to activate prochymosin to chymosin.
9. The process as claimed in claim 8 wherein the filtrate obtained after the addition of sodium benzoate solution is treated with a solution of sodium chloride under stirring and cooking, and the precipitate suspended in a chilled solution of 0.2M glycine with 0.001M EDTA and thereafter treated with 0.23% solution of sodium benzoate and stored under cooling.
10. The process as claimed in claim 9 wherein said chymosin obtained is formulated with 10% of sodium chloride and 0.2% of Trehalose.

## 11. Recombinant calf-chymosin having the following amino acid sequence:

MetAlaSerIle ThrArgIle ProLeuTyr LysGlyLysSer LeuArgLys AlaLeuLys  
 1 ATGGCTAGCA TCACTAGGAT CCCTCTGTAC AAAGGCAAGT CTCTGAGGAA GGCGCTGAAG  
 TACCGATCGT AGTGATCCTA GGGAGACATG TTTCCGTTCA GAGACTCCTT CCGCGACTTC  
 GluHisGlyLeu LeuGluAsp PheLeuGln LysGlnGlnTyr GlyIleSer SerLysTyr  
 61 GAGCATGGGC TTCTGGAGGA CTTCTGCAG AACAGCAGT ATGGCATCAG CAGCAACTAC  
 CTCGTACCGG AAGACCTCCT GAAGGACGTC TTTGTCGTCA TACCGTAGTC GTCGTTCATG  
 SerGlyPheGly GluValAla SerValPro LeuThrAsnTyr LeuAspSer GlnTyrPhe  
 121 TCCGGCTTCG GGGAGGTGGC CAGCGTGCCC CTGACCAACT ACCTGGATAG TCAGTACTTT  
 AGGCCGAAGC CCCTCCACCG GTCGCACGGG GACTGGTTGA TGGACCTATC AGTCATGAAA  
 GlyLysIleTyr LeuGlyThr ProProGln GluPheThrVal LeuPheAsp ThrGlySer  
 181 GGGAAGATCT ACCTCGGGAC CCCGCCCCAG GAGTTCACCG TGCTGTTTGA CACTGGCTCC  
 CCCTTCTAGA TGGAGCCCTG GGGCGGGGTC CTCAAGTGGC ACGACAAACT GTGACCGAGG  
 SerAspPheTrp ValProSer IleTyrCys LysSerAsnAla CysLysAsn HisGlnArg  
 241 TCTGACTTCT GGGTACCCTC TATCTACTGC AAGAGCAATG CCTGCAAAAA CCACCAGCGC  
 AGACTGAAGA CCCATGGGAG ATAGATGACG TTCTCGTTAC GGACGTTTTT GGTGGTCGCG  
 PheAspProArg LysSerSer ThrPheGln AsnLeuGlyLys ProLeuSer IleHisTyr  
 301 TTCGACCCGA GAAAGTCGTC CACCTTCCAG AACCTGGGCA AGCCCCTGTC TATCCACTAC  
 AAGCTGGGCT CTTTCAGCAG GTGGAAGGTC TTGGACCCGT TCGGGGACAG ATAGGTGATG  
 GlyThrGlyLys MetGlnGly IleLeuGly TyrAspThrVal ThrValSer AsnIleVal  
 361 GGGACAGGCA AGATGCAGGG GATCCTGGGC TATGACACCG TCACTGTCTC CAACATTGTG  
 CCCTGTCCGT TCTACGTCCC CTAGGACCCG ATACTGTGGC AGTGACAGAG GTTGTAAACAC  
 AspIleGlnGln ThrValVal LeuSerThr GlnGluProGly AspValPhe ThrTyrAla  
 421 GACATCCAGC AGACAGTAGT CCTGAGCACC CAGGAGCCCC GGGACGTCTT CACCTATGCC  
 CTGTAGGTCG TCTGTCATCA GGACTCGTGG GTCCTCGGGC CCCTGCAGAA GTGGATACGG  
 GluPheAspGly IleLeuGly MetAlaTyr ProSerLeuAla SerGluVal LeuAspThr  
 481 GAATTCGACG GGATCCTGGG GATGGCGTAC CCCTCGCTGG CCTCAGAAGT ACTCGATACC  
 CTTAAGCTGC CTTAGGACCC CTACCGCATG GGGAGCGACC GGAGTCTTCA TGAGCTATGG  
 GlyPheAspAsn MetMetAsn ArgHisLeu ValAlaGlnAsp ValPheSer ValTyrMet  
 541 GGCTTTGACA ACATGATGAA CAGGCACCTG GTGGCCCAAG ACGTGTTCTC GGTTTACATG  
 CCGAAACTGT TGTACTACTT GTCCGTGGAC CACCGGGTTC TGCACAAGAG CCAAATGTAC  
 AspArgAsnGly GlnGlyAsn MetPheThr LeuGlyAlaIle AspProSer TyrTyrThr  
 601 GACAGGAATG GGCAGGGAAA CATGTTTACC CTGGGGGCCA TCGACCCGTC CTACTACACA  
 CTGTCCTTAC CCGTCCCTTT GTACAAATGG GACCCCCGGT AGCTGGGCAG GATGATGTGT  
 GlySerLeuHis TrpValPro ValThrVal GlnGlnTyrTrp GlnPheThr ValAspSer  
 661 GGGTCCCTGC ACTGGGTGCC CGTGACAGTG CAGCAGTACT GGCAGTTCAC TGTGGACAGT  
 CCCAGGGACG TGACCCACGG GCACTGTCAC GTCGTATGA CCGTCAAGTG ACACCTGTCA  
 ValThrIleSer GlyValVal ValAlaCys GluGlyGlyCys GlnAlaIle LeuAspThr  
 721 GTCACCATCA GCGGTGTGGT TGTGGCCTGT GAGGGTGGCT GTCAGGCCAT CCTGGACACG  
 CAGTGGTAGT CGCCACACCA ACACCGGACA CTCCCACCGA CAGTCCGGTA GGACCTGTGC  
 GlyThrSerLys LeuValGly ProSerSer AspIleLeuAsn IleGlnGln AlaIleGly  
 781 GGCACCTCCA AGCTGGTCGG GCCCAGCAGC GACATCCTCA ACATCCAGCA GGCCATTGGA  
 CCGTGGAGGT TCGACCAGCC CGGGTCGTCTG CTGTAGGAGT TGTAGGTCGT CCGGTAACCT  
 AlaThrGlnAsn GlnTyrAsp GluPheAsp IleAspCysAsp AsnLeuSer TyrMetPro  
 841 GCCACACAGA ACCAGTACGA TGAGTTTGAC ATCGACTGCG ACAACCTGAG CTACATGCC  
 CCGTGTGTCT TGGTCATGCT ACTCAAATG TAGCTGACGC TGTGGACTC GATGTACGGG  
 ThrValValPhe GluIleAsn GlyLysMet TyrProLeuThr ProSerAla TyrThrSer  
 901 ACTGTGGTCT TTGAGATCAA TGGCAAAATG TACCCACTGA CCCCTCCGC CTATACCAGC  
 TGACACCAGA AACTCTAGTT ACCGTTTTAC ATGGGTGACT GGGGGAGGCG GATATGGTCG  
 GlnAspGlnGly PheCysThr SerGlyPhe GlnSerGluAsn HisSerGln LysTrpIle

961 CAGGACCAGG GCTTCTGTAC CAGTGGCTTC CAGAGTGAAA ATCATTCCCA GAAATGGATC  
GTCCTGGTCC CGAAGACATG GTCACCGAAG GTCTCACTTT TAGTAAGGGT CTTTACCTAG  
LeuGlyAspVal PheIleArg GluTyrTyr SerValPheAsp ArgAlaAsn AsnLeuVal  
1021 CTGGGGGATG TTTTCATCCG AGAGTATTAC AGCGTCTTTG ACAGGGCCAA CAACCTCGTG  
GACCCCTAC AAAAGTAGGC TCTCATAATG TCGCAGAAAC TGTCCCGGT GTTGGAGCAC  
GlyLeuAlaLys, AlaIle\*\*\*  
1081 GGGCTGGCCA AAGCCATCTG A  
CCCGACCGGT TTCGGTAGAC T

13. Recombinant calf-chymosin when produced by a process according to any of the preceding claims.